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For : PROSTATE-SPECIFIC MEMBRANE ANTIGEN AND USES THEREOF

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Commissioner for Patents
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Alexandria, VA 22313-1450

Sir:

PRELIMINARY AMENDMENT AND INFORMATION DISCLOSURE STATEMENT

Please amend the above-identified application as follows:

Amendments to the Specification:

Please amend the specification under the provisions of 37 C.F.R. §1.121 (revised Amendment format) as indicated below, with added matter indicated by underlining and deleted matter indicated by strikethrough.

Please amend the paragraph on page 91, lines 30-33 as follows:

--PSM is a type two membrane protein. Most type two membrane proteins are binding proteins, transport proteins or peptidases. Prostate Specific Membrane Antigen has activity as a carboxypeptidase and acts on both gamma linked or alpha linked amino acids which have acidic amino acids such as glutamate in the carboxy terminus.--

Please amend the paragraph running from page 91, line 35 to page 92, line 7 as follows:

--Prostate specific membrane antigen is found in high concentration in the seminal plasma. When examining LNCaP cells, PSM antigen has enzymatic activity with N-acetylaspartylglutamate as a substrate and enzymatic action results in the release of, N-acetylaspartate and glutamic acid. In vitro translated PSM message also had this peptidase activity. Because PSM action will release glutamate, and because it is well known that the seminal fluid is highly enriched in its content of glutamic acid, the action of PSM antigen of endogenous protein/peptide substrates may be responsible for generating the glutamic acid present.--

On page 92, line 18, please add the following:

--Thus one skilled in this art would be able to design inhibitors to enhance the activity of the non degraded normal substrate if its increased level will have a biologic desired activity. Also biologic activity can be measured to see how it correlates with the level of message. Tissue may be examined for activity directly rather than indirectly using in-situ analysis or immunohistochemical probes. Because there is another gene highly similar on the other arm of chromosome 11 when isolated the expressed cloned genes can be used to determine what the substrate differences are and one may use those substrates for identification of PDM related activity, for example, in circulating cells when looking for metastases.

PSM specific substrates can be designed that could activate pro-drugs at the site of prostate tumor cells to kill those cells.--

Please amend the paragraph on page 93, lines 1-14 as follows:

--Excitatory neurotransmission in the central nervous system (CNS) is mediated predominantly by glutamate receptors. Two types of glutamate receptors have been identified in the human CNS: metabotropic receptors, which serve G-protein coupled second messenger signaling systems, and ionotropic receptors, which serve as ligand gated ion channels. Ionotropic glutamate channels can increase the inward flow of ions such as calcium ions. This can result in the subsequent stimulation of nitric oxide, and nitric oxide modulation of a number of signaling pathways. Nitric oxide has been found to be a major signaling mechanism involved in cell growth and death, response to inflammation, smooth muscle cell contraction, etc. The presence of ionotropic glutamate receptors in human prostate tissue was investigated.--

Please amend the paragraph on page 93, lines 16-19 as follows:

--Methods: Detection of glutamate receptor expression was performed using anti-gluR2/3 and antigluR4 polyclonal antibodies and antibiotin immunohistochemical techniques in paraffin-embedded human prostate tissues. PSM antigen is a neurocarboxypeptidase that acts to release glutamate. In the CNS glutamate acts as a neurotransmitter by acting on glutaminergic ion channels and increases the flow of ions like calcium ions. One way the glutamate signal is transduced into cell activity is the activation of nitric oxide synthase, and nitric oxide synthase has recently been found to be present in human prostatic tissue. NO is a major signalling mechanism and is involved in control of cell growth and death, in response to inflammation, in smooth muscle cell contraction, etc. In the prostate much of the stroma is smooth muscle. It was discovered that the prostate is rich in glutaminergic receptors and we have begun to define this

relationship. Stromal abnormalities are the key feature of BPH. Stromal epithelial interactions are of importance in both BPH and CaP. The other glutaminergic receptors through G proteins to change the metabolism of the cell. --

Please amend the paragraph running from page 93, line 29 to page 94, line 2 as follows:

--Discussion: PSM antigen is a neurocarboxypeptidase that acts to release glutamate from NAAG 1, also as a potential nerotransmitter. In the CNS glutamate acts as a neurotransmitter by acting on glutaminergic ion channels and increases the flow of ions such as calcium ions. One way the glutamate signal is transduced into cell activity is the activation of nitric oxide synthase, and nitric oxide synthase has recently been found to be present in human prostatic tissue. NO is a major signaling mechanism and is involved in control of cell growth and death, in response to inflammation, in smooth muscle cell contraction, etc,. In the prostate much of the stroma is smooth muscle. The prostate is rich in glutaminergic receptors. Stromal abnormalities are the key feature of BPH. Stromal epithelial interactions are of importance in both BPH and CaP. The other glutaminergic receptors through G proteins to change the metabolism of the cell. Glutamate can be produced in the cerebral cortex through the carboxypeptidase activity of the prostatespecific membrane antigen (PSMA). In this location, PSMA cleaves glutamate from acetyl-aspartyl-glutamate. Taken together, these observations suggest a function for PSMA in the human prostate; glutamate may be an autocrine and/or paracrine signalling molecule, possibly mediating epithelial-stromal interactions. Ionotropic glutamate receptors display a unique compartmental distribution in the human prostate. ~~Distribution~~

The differential distribution of ionotropic glutamate receptor subtypes between the stromal and epithelial compartments of

~~receptors in~~ the prostate has not been described. Prostate-specific membrane antigen(PSMA) has an analogous prostatic distribution, with expression restricted to the epithelial compartment. Basal cells are considered the precursor cell for the prostatic acinar and neuroendocrine cells of the prostate. Glutamate receptors may provide signaling functions in their interactions with the prostate stroma and acinar cells, and PSM may be involved in that interaction. Thus inhibition or enhancement of PSM activity could serve to modulate activity of the basal cells and prove to be a valuable aid for controlling basal cell function in the prostate.--

On page 94, line 21, please add the following:

--PSM carboxypeptidase may serve to process neuropeptide transmitters in the prostate. Neuropeptide transmitters are associated with the neuroendocrine cells of the prostate and neuroendocrine cells are thought to play a role in prostatic tumor progression. Interestingly, PSM antigen's expression is upregulated in cancer. Peptides known to act as prostatic growth factors such as TGF-a and bFGF, up regulate the expression of the antigen. TNF on the other hand downregulate PSM. TGF and FGF act through the mitogen activated signaling pathway, while TNF acts through the stress activated protein kinase pathway. Thus modulation of PSM expression is useful for enhancing therapy.--

Please amend the paragraph on page 94, lines 22-28 as follows:

--Because of PSM's carboxypeptidase-like activity and due to the fact that one of its substrates is the dipeptide N-acetyl-aspartyl glutamic acid, NAAG, which is one of the best substrates found to date to act as a neurotransmitter in the central nervous system, ~~A~~altering PSM antigen function may also have beneficial actions outside the prostate. In the rat CNS a protein homology

to PSM antigen was discovered and provides a rational to consider prostate specific membrane antigen as a neurocarboxypeptidase. Abnormalities or ~~A~~alterations in its function may occur in neurotoxic disorders such as epilepsy, or ALS, alzheimers, and multiple sclerosis. --

Please amend the paragraph on page 103, lines 2-21 as follows:

--Membrane-bound PSM antigen has pteroyl poly gamma-glutamyl carboxypeptidase (folate hydrolase) activity. Prostate specific membrane antigen was immuno precipitated from the prostate cancer cell line LNCaP and demonstrated it to be rich in folate hydolase activity, with gammaglutamated folate or polyglutamated methotrexate being much more potent inhibitors of the neuropeptidase activity than was quisqualate, which was the most potent inhibitor reported up to this time and consistent with the notion that polyglutamated folates may be the preferred substrate. Gamma-glutamyl hydrolase activity is also present in lysosomes of cells and these enzymes may be responsible for regulating the length of exogenous and endogenous folyl polyglutamate chain lengths. A characteristic difference between these two hydrolases is that the PSM enzyme exhibits substantial activity at pH values 7.5 to 8.0 in addition to having an acidic pH 4.5 to 5 optimum. Moderate levels of hydrolase activity are present within LNCaP cytosolic compartment and may represent the short intracellular fragment of this class II enzyme. This reflects an interesting situation in these cells where the majority of RNA codes for the membrane-bound enzyme that is localized extracellularly. The ratio of the mRNAs in these samples that code for the class II membrane and the cytosolic proteins is ten to one. In normal prostate tissue, the mRNA coding for the membrane protein is only one-tenth that of the cytosolic form.--

Please amend the paragraph on page 105, lines 7-18 as follows:

--PSM folate hydrolase activity can possibly be used as a prodrug converting enzyme. Prodrugs may be generated which would activate at the site of the tumor such as N-phosphonoacetyl-1-aspartate-glutamate. PALglu is an inhibitor of the enzyme activity with NAAG as a substrate. In the normal prostate PSM is intracellular. In the transformed cell the majority of the protein and its attendance enzymatic activity is extracellular in location. It may be that as the enzymes associated with cell growth require the polyglutamated forms the cancer finds a way to remove PSM folate hydrolase from the interior by alternative splicing to an extracellular enzyme. PSM is a membrane protein and is found to predominate in cancer, but PSM' is likely a cytosolic protein which predominates in the normal condition.--

On page 105, line 24, please add the following:
-- For the cytotoxic drug methotrexate to be a tumor toxin it has to get into the cell and be polygammaglutamated to be active, because polyglutamated forms serve as the enzyme substrates and because polyglutamated forms or toxins are also retained by the cell. Folate hydrolase is a competing reaction and deglutamates methotrexate which then can diffuse back out of the cell. Cells that overexpose folate hydrolase activity are resistant to methotrexate. Prostate cancer has always been absolutely refractory to methotrexate therapy and this may explain why, since the prostate and prostate cancer has a lot of folate hydolase activity.--

On page 108, line 4, please add the following:

--Penta-gammaglutamyl-folate is a very potent inhibitor of activity (inhibition of the activity of the enzyme is with 0.5um Ki.) As penta-gammaglutamyl-folate may also be a substrate and as

folates have to be depolygammaglutamated in order to be transported into the cell, this suggests that this enzyme may also play a role in folate metabolism. Folate is necessary for the support of cell function and growth and thus this enzyme may serve to modulate folate access to the prostate and prostate tumor. The other area where PSM is expressed is in the small intestine. It turns out that a key enzyme of the small intestine that is involved in folate uptake acts as a gammacarboxypeptidase in sequentially proteolytically removing the terminal gammaglutaminyl group from folate. In the bone there is a high level of unusual gammaglutamate modified proteins in which the gamma glutamyl group is further carboxylated to produce gammacarboxyglutamate, or GLA. One such protein is osteonectin.

Using capillary electrophoresisis pteroyl poly-gammaglutamate carboxypeptidase (hydrolase) activity was investigated in membrane preparations from androgen sensitive human prostatic carcinoma cells (LNCaP). The enzyme immunologically cross-reacts with a derivative of an anti-prostate monoclonal antibody (7E11-C5) that recognizes prostate specific membrane (PSM) antigen. The PSM enzyme hydrolyzes gamma-glutamyl linkages and is an exopeptidase as it liberates progressively glutamates from methotrexate triuglutamate ($MTXGlu_3$) and folate pentaglutamate ($Pte\ Glu_3$) with accumulation of MTX and Pte Glu respectively. The semi-purified membrane-bound enzyme has a broad activity from pH 2 to 10 and is maximally active at pH4.0 Enzymatic activity was weakly inhibited by dithfothreitol ($\geq$0.2 mM) but not by reduced glutathione, homocysteine, or p-hydroxymercuribenzoate (0.05 mM). By contrast to LNCaP cell membranes, membranes isolated from androgen insensitive human prostate (TSU-Prl, Duke-145, PC-3) and estrogen-sensitive mammary adenocarcinoma (MCF-7) cells do not exhibit comparable hydrolase activity nor do they react with 7E11-C5. Thus, a folate hydrolase was identified in LNCap cells that exhibits exopeptidase activity and is strongly expressed by these cells.

PALA-Glutamate 3 was tested for efficacy of the prodrug strategy by preparing N-acetylaspartylglutamate, NAAG 1(Figure 33). NAAG was synthesized from commercially available gamma-benzylaspartate which was acetylated with acetic anhydride in pyridine to afford N-acetylgamma-benzyl aspartate in nearly quantitative yield. The latter was activated as its pentafluorophenyl ester by treatment with pentafluorophenyltrifluoroacetate in pyridine at 0 deg.C for an hour. This activated ester constitutes the central piece in the preparation of compounds 1 and 4 (Figure 34). When 6 is reacted with epsilon-benzyl-L-glutamate in the presence of HOAT(1-hydroxy azabenzotriazole) in THF-DMF (tetrahydrofuran, N,N- dimethylformamide) at reflux for an overnight period and after removal of the benzyl protecting groups by hydrogenolysis (H2, 30 psi, 10% Pd/C in ethylacetate) gave a product which was identical in all respects to commercially available NAAG (Sigma).--

On page 109, line 33, please add the following:

--In addition, most if not all chemotherapies rely on one hypothesis; fast growing cells possess a far higher appetite for nutrients than normal cells. Hence, they uptake most of the chemotherapeutic drugs in their proximity. This is why chemotherapy is associated with serious secondary effects (weakening of the immune system, loss of hair,...) that sometimes put the patient's life in danger. A selective and effective drug that cures where it should without damaging what it shouldn't damage is embodied in representative structures 21 and 22. --

Please amend the paragraph on page 111, lines 17-24 as follows:

--In the latter, a compound like 27 when attached to a multiply charged dipeptide like NAAG, has no chance of crossing the blood brain barrier. In the former case, PSM homolog concentration in the small intestines is in the brush border and is low compared

to that of PSM in the prostate cancer cells and is thus not likely to be exposed to prodrugs in the serum. In addition, one could enhance the selectivity of delivery of the prodrug by local injection in the prostate. Another image of this strategy could be formulated as follows. If prostate cancer were a war in which one needed a "smart bomb" to minimize the damage within the peaceful surroundings of the war zone, then 21 would be that "smart bomb". NAAG would be its guidance system, PSM would be the trigger, and 27 would be the warhead. --

Please amend the paragraph on page 111, lines 26-35 as follows:

--26 and its analogs are established active molecules that portray the activity of dynemycin A. Their synthesis is described in the literature. The total synthesis of optically active 27 has been described[6]. The synthetic scheme that for the preparation of 28 is almost the same as that of 27. However, they differ only at the position of the methoxy group which is meta to the nitrogen in the case of 28. This requires an intermediate of type 29 prepared by modification of the Myers' method. Compound 28 is perhaps the closest optically active analog to 26, and the activity of the latter is known and very high.--

Please amend the paragraph on page 112, lines 19-28 as follows:

--Intramolecular assisted hydrolysis of systems like N-acetylaspartyle is well documented in the literature. The aminoacid portion should facilitate the hydrolysis of such a linkage. In the event this would not work when NAAG is placed on the nitrogen, an alternative would be to attach NAAG to the oxygen giving rise to phenolic ester 22 which is per se labile and removable under milder conditions. PSM specific pro-drugs can be designed that could activate pro-drugs at the site of

prostatic tumor cells to kill those cells. PSM specific substrates may also be used in the treatment of benign prostatic hyperplasia.--

In the claims:

Please amend as follows:

Please cancel claims 1-23 without prejudice or disclaimer.

Please add the following new claims to the application:

--24. (New) A method comprising administering an inhibitor of the neurocarboxypeptidase activity of prostate specific membrane antigen so as to inhibit release of glutamate by N-acetylaspartylglutamic acid (NAAG) hydrolysis.--

--25. (New) The method of claim 24, wherein the inhibitor is $MTXglu_3$, $pteglu_5$ or $pABAglu_5$.

REMARKS

In the present Preliminary Amendment, claims 1-23 of the application are cancelled without prejudice or disclaimer, and replaced by new claims 24-25.

Support for these new claims is found, inter alia, in the specification as follows: Claim 24: page 92, lines 22-30 and page 102, lines 29-36, and Claim 25: page 102, lines 34-36.

These new claims, 24-25, therefore raise no issue of new matter and it is respectfully requested that they be entered into the file of the application.

Applicants note that new claims 24-25 embrace subject matter which is also claimed in U.S. Patent No. 6,413,948 issued July 2, 2002. As required by 35 U.S.C. §135(b), applicants are presenting claims to such subject matter not more than one year after the issue date of U.S. Patent No. 6,413,948. A copy of U.S. Patent No. 6,413,948 is attached hereto as **Exhibit 1.**

Information Disclosure Statement

In accordance with their duty of disclosure under 37 C.F.R. §1.56, applicants direct the Examiner's attention to the following reference which is listed on the PTO-1449 form attached hereto as **Exhibit A**. A copy of this reference is attached hereto as Exhibit 1.

U.S. Patent No. 6,413,948, issued July 2, 2002 to Slusher, et al. (Exhibit 1).

If a telephone conference would be of assistance in advancing the progress of this application, applicants' undersigned attorney invites the Examiner to telephone either of them at the number provided below.

No fee is deemed necessary with the filing of this Preliminary Amendment. However, if any fee is due, authorization is hereby given to charge the required fee to Deposit Account No. 03-3125.

Respectfully submitted,

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